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Structure of the reaction center from *Rhodobacter sphaeroides* R-26 and 2.4.1: Symmetry relations and sequence comparisons between different species*

(bacterial photosynthesis/membrane protein structure/x-ray diffraction)

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ABSTRACT **Photosynthetic reaction centers from purple bacteria exhibit an approximate twofold symmetry axis, which relates both the cofactors and the L and M subunits. For the reaction center from *Rhodobacter sphaeroides*, deviations from this twofold symmetry axis have been quantitated by superposing, by a 180° rotation, the cofactors of the B branch onto the A branch and the M subunit onto the L subunit. An alignment of the sequences of the L and M subunits from four purple bacteria, one green bacterium, and the D_1 and D_2 subunits of a photosystem II-containing green alga is presented. The residues that are conserved in all six species are shown in relation to the structure of *Rb. sphaeroides* and their possible role in the function of the reaction center is discussed. A method is presented for characterizing the exposure of α-helices to the membrane based on the periodicity of conserved residues. This method may prove useful for modeling the three-dimensional structures of membrane proteins.**

The reaction center (RC) is an integral membrane protein–pigment complex that is composed of three subunits L, M, and H and a number of cofactors (four bacteriochlorophylls, two bacteriopheophytins, two quinones, and one iron) (for a review, see ref. 1). In previous papers of this series, we discussed several aspects of the structure of the RC from *Rhodobacter sphaeroides*; the structure has been determined by x-ray diffraction to a resolution of 2.8 Å with an *R* value of 24% (2–6).‖ A striking feature of the structure is the arrangement of the cofactors along two branches (A and B) that are approximately related by a twofold symmetry axis (2, 7). Similarly, the L and M subunits are related to each other by a 180° rotation about the symmetry axis (3, 8). Deviations of the cofactors and subunits from the twofold symmetry give rise to the preferential electron transfer along one branch (A) (reviewed in refs. 5 and 6). In this work, we take a closer look at these deviations by superposing the cofactors of the B branch onto the A branch and the M subunit onto the L subunit. The ability to superpose the two subunits is related to the homology of their residues. The sequences of the L and M subunits of four purple bacteria, *Rb. sphaeroides* (9), *Rhodobacter capsulatus* (10), *Rhodospirillum rubrum* (11), *Rhodopseudomonas viridis* (12); one green bacterium, *Chloroflexus aurantiacus* (13, 14); and the D_1 and D_2 subunits of the green alga, *Chlamydomonas reinhardtii* (15, 16) are compared. The residues that are conserved in all six species are shown in relation to the three-dimensional structure of *Rb. sphaeroides*, and their possible roles in the function of the RC are discussed. The periodicities of the conserved/nonconserved residues of the transmembrane helices have been analyzed and related to the environments of the helices.

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METHODS

Superposition of Symmetry-Related Structures. The coordinate transformation that superposed the RC structure upon itself by a rotation about the local twofold symmetry axis was determined by an algorithm of Kabsch (17). This transformation minimized the rms deviation, Δ, between equivalent C^α atoms in the transmembrane helices of the L and M subunits. A value of 1.27 Å was found for Δ. This same transformation was used to superpose the cofactors of the B branch onto those of the A branch.

Sequence Alignments. Alignment of the sequences of the L and M subunits from *Rb. sphaeroides*, *Rb. capsulatus*, *Rps. viridis*, and the D_1 and D_2 subunits from *C. reinhardtii* were taken from Williams *et al.* (9). Additional sequences from *Rs. rubrum* and *C. aurantiacus* were aligned as presented in refs. 11, 13, and 14.

Fourier Transform Analysis of the Periodicity of Conserved/Nonconserved Residues. The variability index V_j is defined by the number of different types of amino acid residues that are observed at a given position *j* in a family of aligned sequences (for a conserved residue $V_j = 1$). The Fourier transform power spectra, $P(\omega)$, of V_j may be defined in terms of the angular variable ω (rotation angle along a helix axis between residues) by

$$P(\omega) = \left[\sum_{j=1}^{N} (V_j - \bar{V}_j) \cos(j\omega)\right]^2 + \left[\sum_{j=1}^{N} (V_j - \bar{V}_j) \sin(j\omega)\right]^2, \quad [1]$$

where $\bar{V}_j$, is the mean value of V_j, and N is the number of residues in the helix. The parameter ω is related to the number of residues per turn, d, by the expression $\omega = 360°/d$. Similar expressions have been used to describe periodicity in the hydrophobicity profiles of proteins (18, 19). An ideal α-helical pattern can be characterized by a maximum of $P(\omega)$ near $\omega = 100°$ ($d = 3.6$). The number of residues preferentially conserved on one side of a helix can be characterized by the parameter ψ, which is defined by the average value of $P(\omega)$ in

Abbreviations: RC, reaction center; D, bacteriochlorophyll dimer; B, bacteriochlorophyll monomer; ϕ, bacteriopheophytin monomer; Q, quinone; subscripts A and B, branches A and B, respectively.

*This is paper no. 6 in a series. Paper nos. 1–5 are refs. 2–6, respectively.

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‖Coordinates are being prepared for deposition in the Brookhaven Protein Data Bank, Upton, NY 11973.

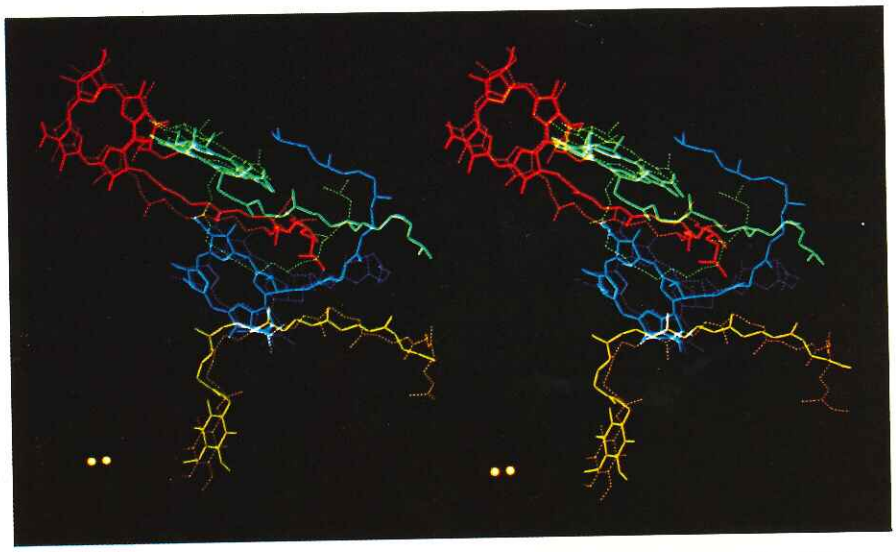

FIG. 1. Stereoview of the cofactors of the B branch (dotted lines) superposed by a 180° rotation about the twofold symmetry axis onto the cofactors of the A branch (solid lines); RC: D (red), B (green), ϕ (blue), Q (yellow), and Fe (small yellow sphere). The Fe does not lie exactly on the symmetry axis; consequently, the transformation shifts its position.

the α-helical region $90° \leq \omega \leq 120°$, relative to the average value of $P(\omega)$:

$$\psi = \left[\frac{1}{30°}\int_{90°}^{120°} P(\omega)\, d\omega\right] \Big/ \left[\frac{1}{180°}\int_{0°}^{180°} P(\omega)\, d\omega\right]. \quad [2]$$

The parameter ψ is bounded by the limits of 0 (no peak) and $180°/30° = 6$ [$P(\omega) \neq 0$ only in the region $90° \leq \omega \leq 120°$]. Values of ψ greater than 2 were taken as indicative of a significant helical pattern in a study of amphipathic helices (19).

RESULTS AND DISCUSSION

Twofold Symmetry Between the Two Cofactor Branches. The superposition of the cofactors of the B branch onto the A branch is presented in Fig. 1. The tetrapyrrole rings of bacteriochlorophyll dimer (D), bacteriochlorophyll monomer (B), and bacteriopheophytin monomer (ϕ) are closely related by the twofold symmetry axis. The largest rms deviations between equivalent atoms are found for the quinones (1.9 Å) and Fe (1.3 Å; i.e., the Fe atom is 0.65 Å on the B side from the twofold axis). Overall, cofactors of the A branch interact more extensively, as reflected in the larger number of van der Waals contacts between cofactors of the A branch. Since electron transfer depends critically on the overlap of the electronic wave functions of the cofactors, small deviations of the cofactor positions may contribute to the preferential electron transfer along the A branch.

Twofold Symmetry Between the L and M Subunits. Another structural source of the asymmetry in electron transfer is the asymmetry of the protein environments of the cofactors. The cofactors interact directly with only two of the three subunits (L and M). To determine the asymmetries between these two subunits the M subunit was rotated onto the L subunit (see Fig. 2). This superposition reveals the striking similarity of the two subunits, particularly in the five transmembrane helices (the rms deviation between equivalent C^{α} atoms is 1.27 Å). The superposition is also close for the periplasmic side of the RC. However, significant differences are seen on the cytoplasmic side, especially the presence of an additional helix, de′, between the D and E helices, and the larger amino-terminal region of the M subunit (3). The cytoplasmic side of the LM complex forms extensive contacts with the H subunit, whose lack of symmetry-related regions reflects the asymmetry of the cytoplasmic region of the LM complex.

Another source of asymmetry is the binding of the carotenoid near the B and C helices of the M subunit in the wild-type strain *Rb. sphaeroides* 2.4.1 (5). Several residues on the B side differ from the corresponding residues on the A side in order to accommodate the carotenoid as well as to provide additional hydrophobic interactions (see Fig. 3).

Interactions involving specific residues that are not conserved between subunits appear to lead to deviations from the twofold symmetry of the cofactors. In particular, the positions of the superposed phytyl chains show large differences (Fig. 1). The position of the phytyl chain of B_B is constrained by a hydrogen bond between the side chain of Ser L178 and the propionic acid group of ring IV (5). In contrast, the phytyl chain of B_A, whose positioning is much different,

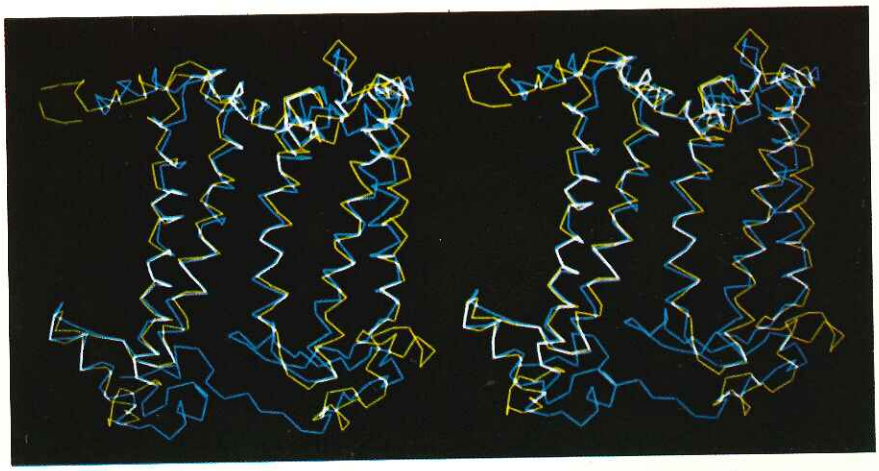

FIG. 2. Stereoview of the backbone of the M subunit (blue) superposed by a 180° rotation about the twofold symmetry axis onto the backbone of the L subunit (yellow). The cytoplasmic side of the subunits is at the bottom. Backbone regions of the two subunits at the same position appear white.

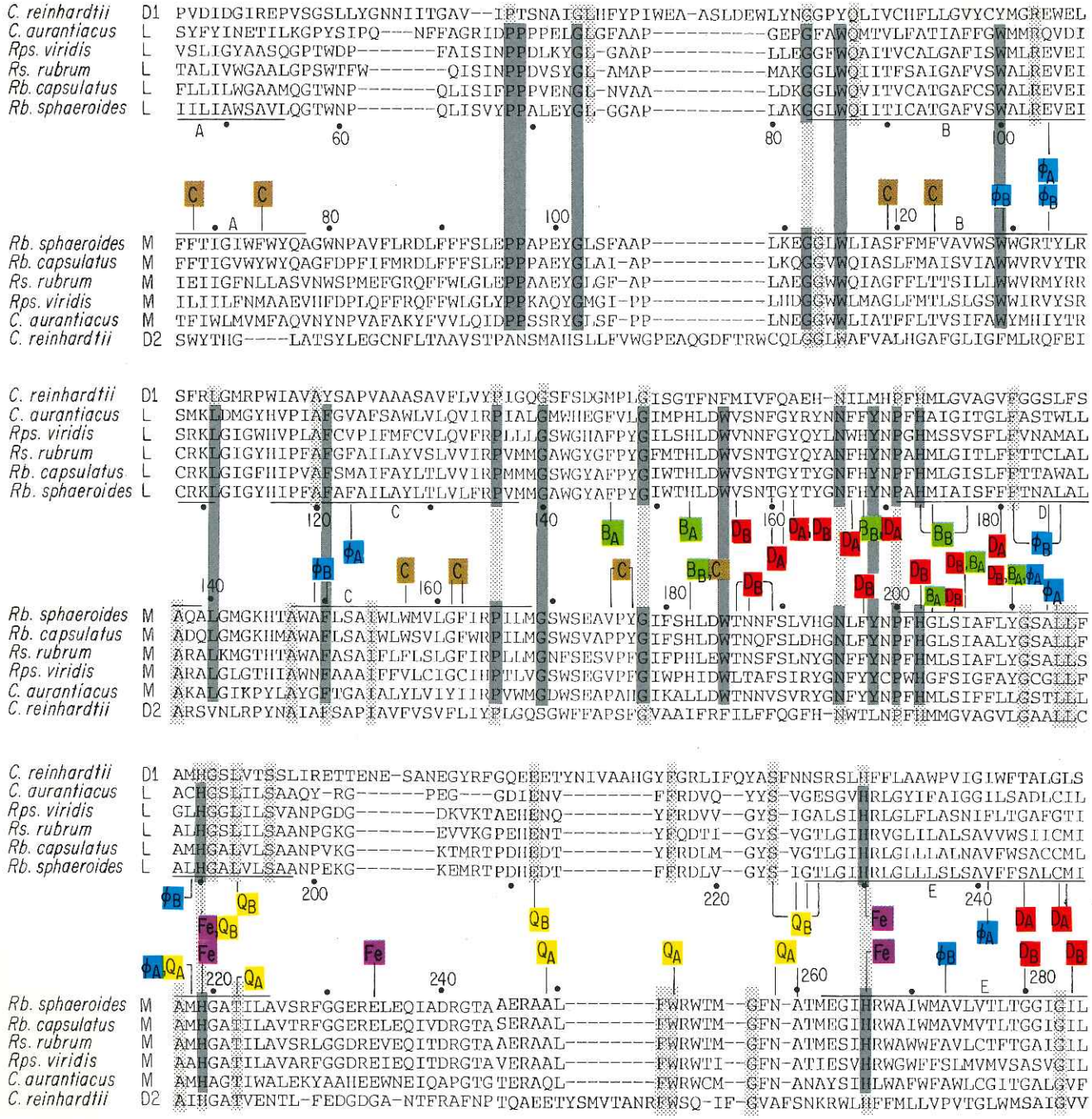

FIG. 3. Comparison of the L and M sequences from the bacteria *Rb. sphaeroides*, *Rb. capsulatus*, *Rs. rubrum*, *Rps. viridis*, *C. aurantiacus*, and the D_1 and D_2 sequences from the green alga, *C. reinhardtii*. The amino- and carboxyl-terminal segments of the sequences have been omitted. Sequence numbers refer to the L and M sequences from *Rb. sphaeroides*. Residues forming transmembrane helices in the L and M subunits of *Rb. sphaeroides* are underlined and labeled A–E. Cofactors (excluding the phytyl and isoprenoid chains) in contact (within 3.5 Å) with residues of *Rb. sphaeroides* are shown in colored squares: D (red), B (green), ϕ (blue), Q (yellow), Fe (purple), and carotenoid, C (brown). Residues that are conserved in all six species of either L and D_1 or M and D_2 (or in all 12 sequences) are stippled; residues that are conserved in both the L and M subunits of the 5 bacterial species (i.e., 10 sequences) are gray.

is not restricted by such a bond to the symmetry-related residue Ala M207 (5). Also, the path of the phytyl chain of ϕ_B is more restricted by aromatic residues than that of ϕ_A. For example, Trp M66 is near ϕ_B; the corresponding residue near ϕ_A is Gly L45 (not shown in Fig. 3; see ref. 9).

Sequence Comparison of Different Photosynthetic Systems. Residues that are important to the function of RCs are likely to be conserved between different species. Based on the similarity between sequences, the L and M subunits in bacterial RCs have been proposed to be homologous to the D_1 and D_2 subunits of photosystem II in green algae, cyanobacteria, and plant systems (9, 10, 20, 21). The aligned sequences of the L and M subunits from five bacterial species and of the D_1 and D_2 subunits in a green plant system are shown in Fig. 3. Twenty-three residues are conserved between the L/D_1 chains of all six species and 23 residues are conserved between the M/D_2 chains, for the region presented in Fig. 3 (stippled regions).

The conserved residues near the cofactors are presented in Fig. 4. Most striking is the limited number of conserved residues near the tetrapyrrole rings. His L173 and M202, near the Mg of D, and Phe L181, bridging D and ϕ_B, are conserved

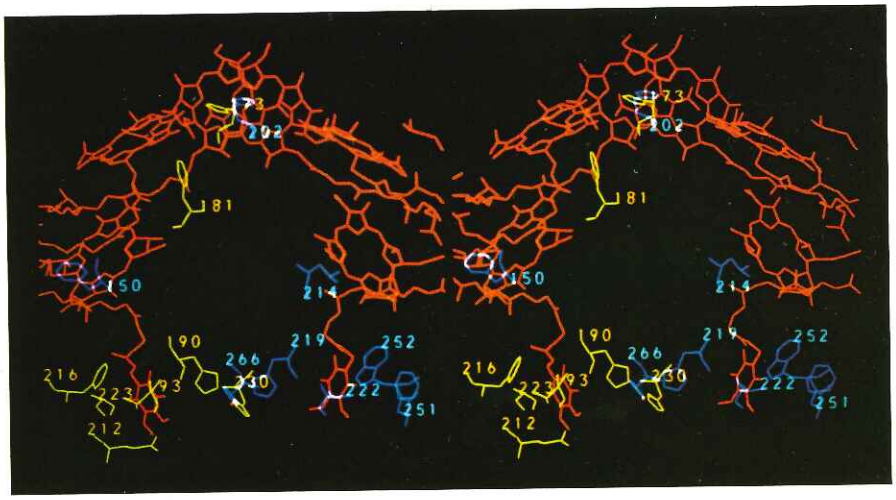

FIG. 4. Stereoview of cofactors (red) and nearby residues (L subunit, yellow; M subunit, blue) that are conserved between all six species (see stippled region in Fig. 3): Glu L212, His (L173, L190, L230, M202, M219, M266), Leu (L193, M214), Phe (L181, L216, M150, M251), Ser L223, Thr M222, and Trp M252. The twofold symmetry axis is approximately in the plane of the figure. The cytoplasmic side is at the bottom.

in all six species. However, His M182 near the Mg of B, Tyr M210, bridging D_B and ϕ_A, and Glu L104 near ϕ_A are conserved in the four purple bacteria; His L153 near B_A is conserved in the five bacterial species. None of the residues in contact with the carotenoid are conserved in all six species; only Gly M161 and His M182 are conserved among purple bacteria (see Fig. 3).

Several residues near the quinones (Q) are conserved in all six species. All residues whose side chains form hydrogen bonds with the quinones—i.e., Thr M222, Ser L223, and His L190—are conserved (Q_A also forms a hydrogen bond with a backbone nitrogen of M260). Other conserved residues near the quinones are three aromatic residues—Phe L216, Phe M251, and Trp M252. Similarly, Glu L212, which may be involved in the protonation of Q_B, is conserved.

Residues that are conserved in all six species near the Fe include the four histidines (L190, L230, M219, M266). Glu M234, which also forms a ligand to Fe, is conserved only in bacteria. This residue is part of the de′ helix of the M subunit (3). There is no counterpart of this helix in the L subunit (see gap between residues L203 and L204 in Fig. 3). However, the D_1 subunit has a de′ helix. To accommodate it in the structure, the de′ helix of D_2 needs to be repositioned relative to the de′ helix of the bacterial M subunit. An Fe ligand could then be provided by other Asp or Glu residues present on the D_1 or D_2 subunits. This would place more carboxylate groups near Fe, making it susceptible to oxidation/reduction reaction as has been observed (22). It is also possible that glutamic acid is replaced by carbonate in plant systems (20, 23).

Many of the conserved residues are not in contact with the cofactors. They may, instead, play a role in stabilizing the tertiary structure of the RC (see Fig. 5). Most are located in the periplasmic and cytoplasmic regions and are positioned near the ends of helices or along loops connecting two helices (see Figs. 3 and 5), as has been noted by Michel and Deisenhofer (20).

The presence of residues that are conserved in all sequences suggests a similarity in the three-dimensional structure between the RCs of these species. The limited number of conserved residues near the tetrapyrrole rings may be due to different characteristics of these cofactors in different species. For example, His M182 is not conserved in *C.*

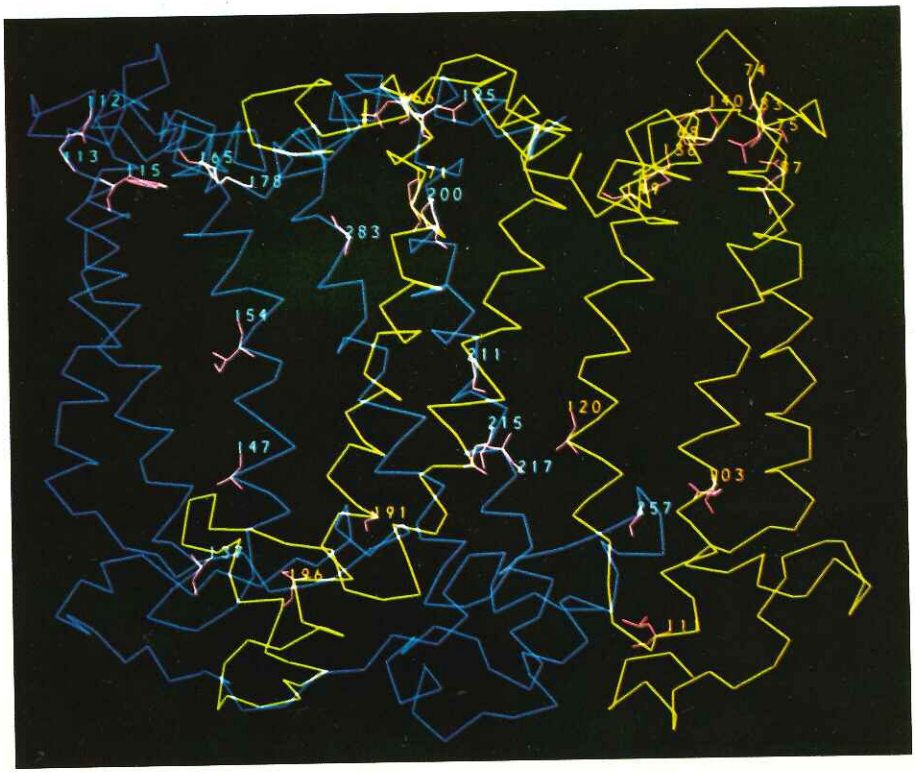

FIG. 5. Backbone of the L subunit (yellow), M subunit (blue), and residues (purple) that are conserved between all six species (see Fig. 3) but are not near cofactors. Residues Ala (L120, M137), Asn (L166, M195), Gly (L74, L83, L140, L149, M112, M113, M178, M257, M283), Leu (L75, L111), and Pro (L68, L136, L171, M165, M200) are positioned either near the end of a helix or in a loop connecting helices. Also shown are Ala (M147, M217), Arg L103, Gln L87, Gly (L191, M211), Ile M154, Leu M215, Ser L196, and Trp M115. View is the same as in Fig. 4.

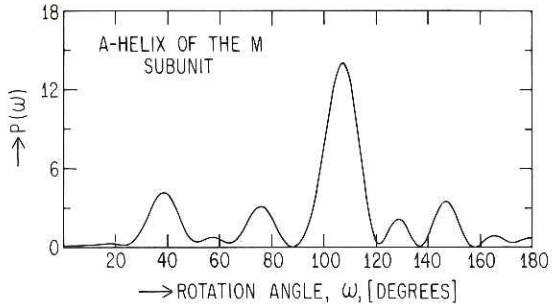

FIG. 6. The Fourier transform spectrum $P(\omega)$ for the transmembrane helices, A, of the M subunits of the 5 bacterial species (Eq. **1**). The peak at $\omega = 105°$ corresponds to a periodicity of 3.4 residues per turn and is consistent with an α-helical conformation. The normalized area of the peak ψ (Eq. **2**) reflects the preferential conservation of residues on one side of the helix.

aurantiacus. This difference may be responsible for the substitution of one bacteriochlorophyll monomer with a bacteriopheophytin in *C. aurantiacus* (13, 24).

From the percentage identity of sequences, one can estimate the similarities of the corresponding structures. Chothia and Lesk (25) obtained an empirical relation:

$$\Delta(\text{Å}) = 0.40\ e^{1.87H}, \qquad [3]$$

where Δ is the rms deviation in the position of the backbone atoms, and H is the percentage identity between sequences. For example, the percentage identity between the transmembrane helices of the L and M subunits of *Rb. sphaeroides* is 27%; Eq. **3** predicts an rms deviation of 1.56 Å, in fair agreement with the value of 1.27 Å derived from the structure. Using the alignment of Fig. 3, rms deviations of 0.65 Å, 0.74 Å, 1.06 Å, 1.28 Å, and 1.63 Å are predicted between the C^{α} atoms of *Rb. sphaeroides* and *Rb. capsulatus*, *Rs. rubrum*, *Rps. viridis*, *C. aurantiacus*, and *C. reinhardtii*, respectively.

Periodicity of Residues of the Transmembrane Helices. Sequence alignments can provide general structural information in addition to the identification of residues that have specific functional and structural significance. Early analysis of homologous sequences emphasized that surface residues of water-soluble proteins are poorly conserved (26). More detailed analyses have established this trend for both globular (25) and integral membrane proteins (4).

An interesting situation arises when regular secondary structure elements, such as α-helices, are located on the surface of a protein. The residues forming this structure will be exposed to an environment that periodically varies between a protein interior and solvent (or membrane) exterior as a function of sequence number. For example, this periodicity in environment is reflected in the repeating pattern of apolar and polar residues that are found in α-helices on the surface of globular proteins (27). In this work, we demonstrate an analogous periodicity in the pattern of residues that are conserved in α-helical regions of homologous proteins.

Fourier transform calculations provide a method for characterizing the periodicity of conserved residues (28). The Fourier transform spectrum, $P(\omega)$, calculated (Eq. **1**) for the A transmembrane helix of the M subunit is shown in Fig. 6. This spectrum shows a prominent peak centered at $\omega = 105°$, which corresponds to a periodicity of $d = 3.4$ residues per turn. The value is consistent with the α-helical conformation of these residues. The peak position is at a higher value of ω than obtained for α-helices of water-soluble proteins, for which $\omega = 97°$ ($d = 3.7$) (19). The increased value of ω reflects either a more tightly wound helix or a systematic shift in the residues that contact an adjacent helix, whose axis is tilted relative to the other helix axis.

The normalized area of the peak, ψ, provides a measure of the number of residues that are preferentially conserved on one side of the helix. The calculated values of ψ (Eq. **2**) are 2.5, 2.5, 1.5, 1.4, and 0.7 for the transmembrane helices A, B, C, D, and E, respectively. The more peripheral helices A and B have larger ψ values than the core D and E helices. This is consistent with a previous analysis that showed that residues exposed to the membrane are poorly conserved while buried residues are highly conserved (4). These results suggest that ψ provides a measure of the exposure of a helix to the membrane that may prove useful for modeling the three-dimensional structures of membrane proteins based on their primary sequence.

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